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A guide to induced spawning and larval rearing of milkfish *Chanos chanos* (Forsskal)

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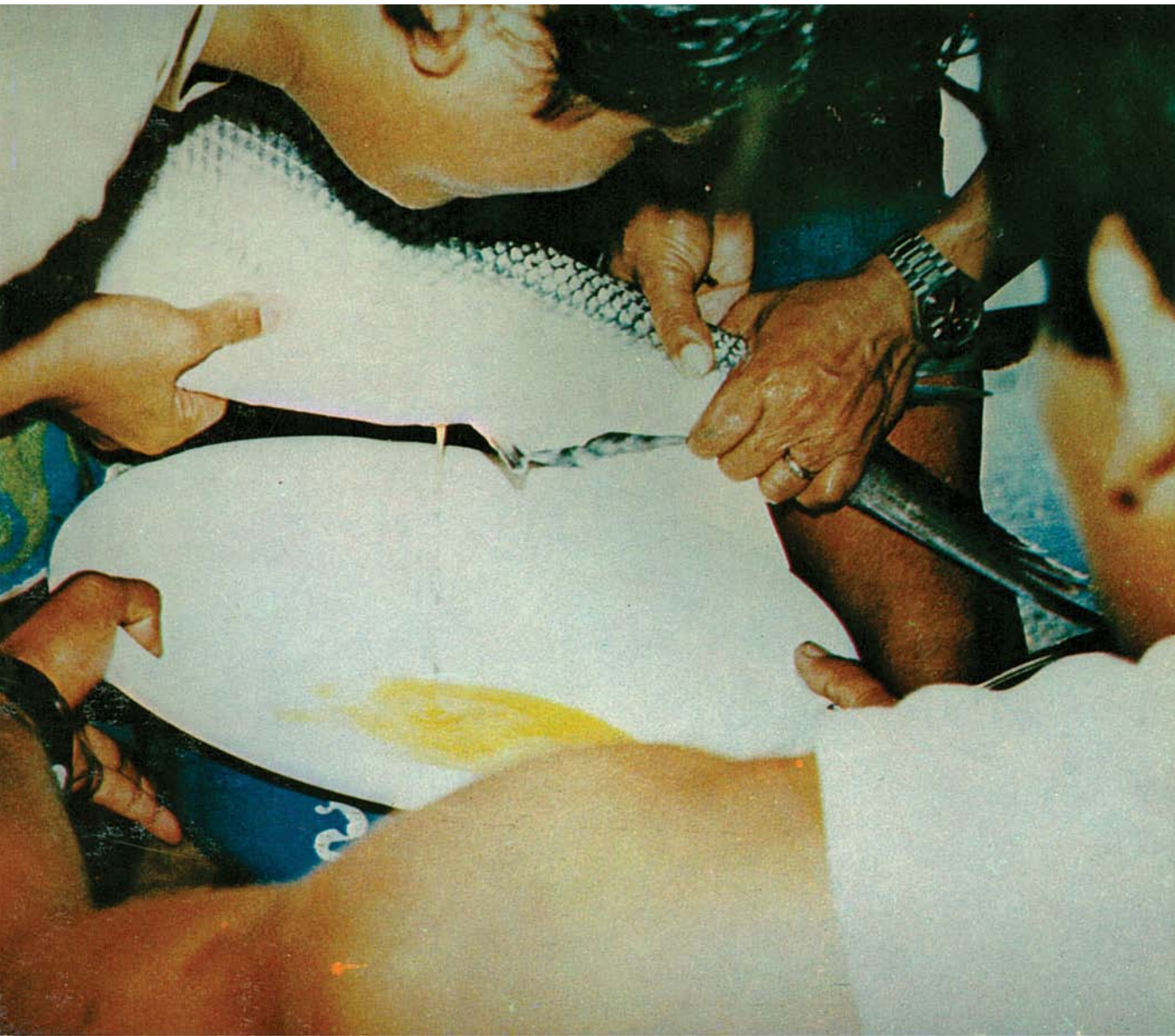

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A Guide to Induced Spawning and Larval Rearing of Milkfish *Chanos chanos* (Forsskal)

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FOREWORD

Milkfish is an important food fish in Indonesia, Taiwan and the Philippines. Although it has been cultured for centuries in brackishwater ponds, fishfarmers are still entirely dependent on fry collected along the coastline during the breeding season. The main constraint to the intensification and expansion of the milkfish industry is an inadequate supply of fry.

There is growing interest in breeding milkfish in captivity because of its importance as food item and the potential of using milkfish fingerlings as bait for the tuna and the skipjack industries. This guide has been written to share the technique developed at SEAFDEC for the artificial propagation of milkfish. The detailed results of our study on the induced breeding and larval rearing of milkfish, which was partially supported by Canada's International Development Research Centre from 1976 to date, have been published elsewhere. It is our hope that this guide will be useful to the fisheries sector in the Philippines, Taiwan, Indonesia and elsewhere.

This guide is definitely not a recipe for success in the artificial propagation of milkfish fry. We expect to revise this guide as additional data become available, especially those related to the mass production of milkfish fry in larger tanks. We request your comments, suggestions, and where necessary, corrections to ensure the usefulness of subsequent editions.

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A GUIDE TO INDUCED SPAWNING AND LARVAL REARING OF MILKFISH *CHANOS CHANOS* (FORSSKAL)

The artificial propagation of milkfish has been attempted in Hawaii, Indonesia, Taiwan, Tahiti and the Philippines; but so far, success has only been achieved in the Philippines (Vanstone *et al.*, 1977; Chaudhuri *et al.,* 1977, 1978; Liao *et al.,* 1979; Juario and Natividad, 1980) and in Taiwan (Tseng and Hsiao, 1979). The induced spawning and larval rearing techniques discussed in this guide has given us consistently good results with both wild and captive spawners during the 1978 to 1983 spawning seasons.

Capture and transport of spawners

The adult milkfish in the fish corral, should be caught with a scoop net lined with a fine-mesh net. If the trap is near the research station, the fish can be transported in a floating cage hitched underneath the outrigger of a pumpboat and the cage is slowly pulled toward the shore. When only one or two fish are caught, transfer each of them directly into aerated doubled plastic bags containing seawater and transport them to the shore aboard a pumpboat. As soon as the pumpboat reaches the shoreline, carry the fish to the experimental tank on a stretcher. For milkfish caught in traps far away from the experimental station, the fish should be transferred to a 2-m diameter canvas tank aboard a pick-up truck. The tank should be 1/3-full of 18-20 ppt seawater (Vanstone *et al.,* 1976). Aerate the water continuously during transport. Upon reaching the station, transfer the fish immediately to the experimental tank by means of a stretcher (Fig. 1).

Fig. 1. Transport of milkfish spawners by land. A spawner within the doubled plastic bag is being transported to the experimental tank by means of a stretcher.

A maximum of four fish can be transported in a single 2-m diameter canvas tank, but only one fish should be transported per plastic bag.

Adult milkfish from captive broodstock reared in floating cages should be caught by hook and line. This method minimizes stress in other spawners. Stress due to handling will cause atresia in maturing or mature females (Lacanilao and Marte, 1982). In order that the fish will take the bait, do not feed the fish the day prior to catching. Transfer the fish in aerated seawater contained in one section of the hull of the pumpboat. In the absence of aeration, change 1/3 to 1/2 of the water every 30 minutes.

Determination of sex and weight of fish

From the external appearance alone it is not possible to distinguish a male from a female milkfish (Fig. 2). Although generally, males are smaller than females, size is not a reliable criterion for distinguishing the sexes. During the spawning season, the sex of the fish can be determined by aspirating gametes through a fine polyethylene cannula (Clay Adams, PE 160) with an inner diameter of about 1.1 mm.

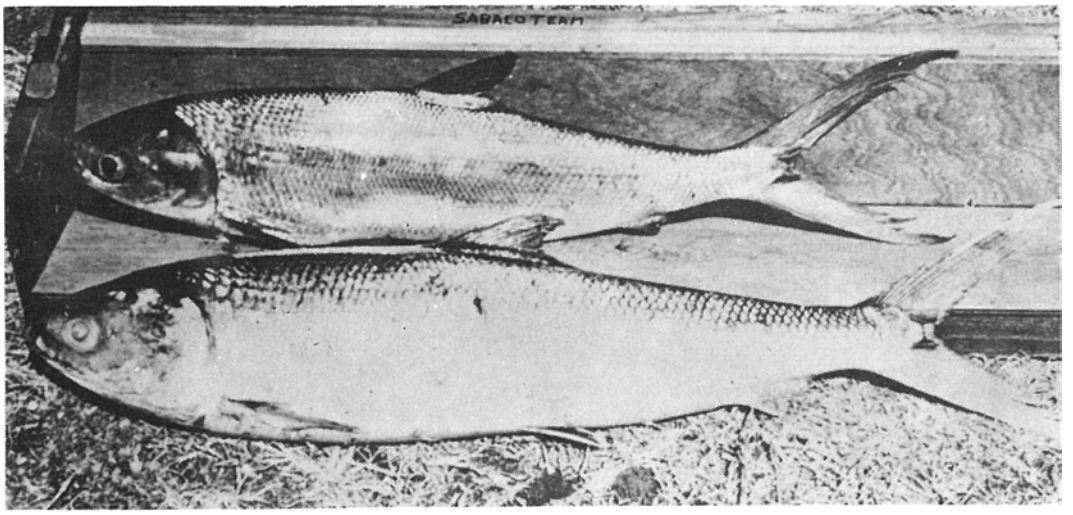


Fig. 2. A male (above) and female (below) wild adult milkfish.

Before aspirating gametes, anesthetize the fish completely by placing it in a doubled plastic bag with seawater containing 100 ppm 2-phenoxyethanol (ethyleneglycol monophenyl ether, Merck). When the fish is immobile, carefully turn it ventral side up. Cautiously insert about 15 to 20 cm of the cannula into the gonads of the fish through the region of the urogenital pore which is located behind the anal opening (Fig. 3). The gametes can be sampled by aspirating them as the cannula is slowly withdrawn (Fig. 4). After egg sampling, measure the fork length of the fish and estimate its weight from a length weight curve; or weigh the fish rapidly but carefully on a platform balance. We usually prefer to only estimate the weight to prevent further stress.

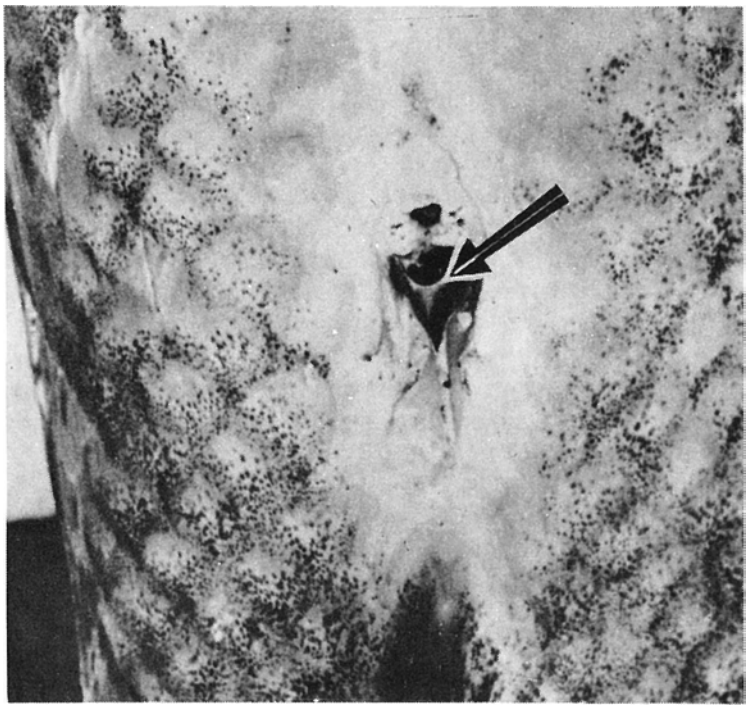


Fig. 3. Ventral view of the posterior portion of a wild adult milkfish showing urogenital region (arrow).

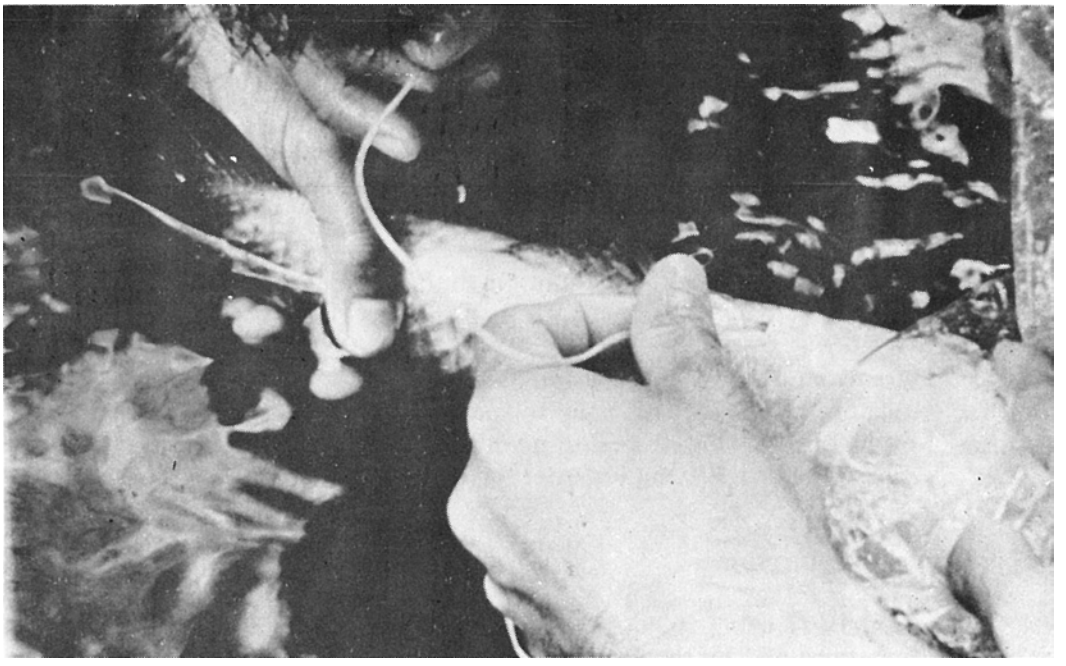


Fig. 4. Sampling of milt/eggs by means of a polyethylene cannula

Determination of maturity

Determine the stage of maturity of individual females from the average diameter of their eggs. Milkfish eggs with a diameter of 0.65 mm or larger are at the tertiary yolk globule or more advanced stages.

Collect some eggs by means of a polyethylene cannula. Place the eggs in a petri dish containing 1% formalin in 0.9% NaCl solution. Measure the diameter of at least 50 eggs under a compound microscope by means of a micrometer eyepiece. If the eggs are not perfectly round, measure the largest and smallest diameter to obtain the average. If the average diameter is equal to or greater than 0.65 mm, the fish is induced to spawn by hormone injection. Fish with an average egg diameter equal to or greater than 0.65 mm can be induced to spawn by hormone injections, while those with an average egg diameter lower than 0.65 mm usually die before completing maturation.

Males are ripe if milt oozes out from the urogenital pore when the abdomen is pressed. If the degree of maturity is right, then milt will be white and creamy; poor milt is watery and curdled. Some mature males caught during the early part of the season may have a very viscous milt (especially true to the captive stock) so that milt does not come out even after pressing the abdomen several times. If this is the case, insert a polyethylene cannula through the urogenital pore to withdraw some milt. Then assess sperm motility and vitality as described by Mounib (1978; Appendix 1). Induce seminal thinning by hormone injection if in the very viscous milt less than 30% of the sperms are motile.

Induced spawning

To induce spawning, use the following hormones:

SPH — acetone dried pituitary gland homogenate of coho salmon prepared by the British Columbia Research Council at Vancouver, Canada; one gram of the powder contains 17.6 mg gonadotropin.

HCG — human chorionic gonadotropin, manufactured by Ayerst Laboratories, New York.

DF — Durandron Forte "250", a long-acting androgen manufactured by N.V. Organon, Holland. This is an oily solution containing 30 mg testosterone propionate, 60 mg testosterone phenylpropionate, 60 mg testosterone isocaproate and 100 mg testosterone decanoate.

Preparation of the injection

Dissolved HCG using its accompanying diluent. The volume of the diluent to be used is determined by the desired concentration of HCG in the 3 ml injection. To avoid possible damage to the muscle tissue, it is recommended that not more

than 3 ml of solution is used for injection. The necessary amount of acetone-dried pituitary gland is homogenized in this solution before it is finally administered. In cases where it becomes necessary to inject more than 3 ml of HCG solution, administer the injection in divided quantities at two different sites on the dorsal musculature, a few centimeters below the dorsal fin (Fig. 5).

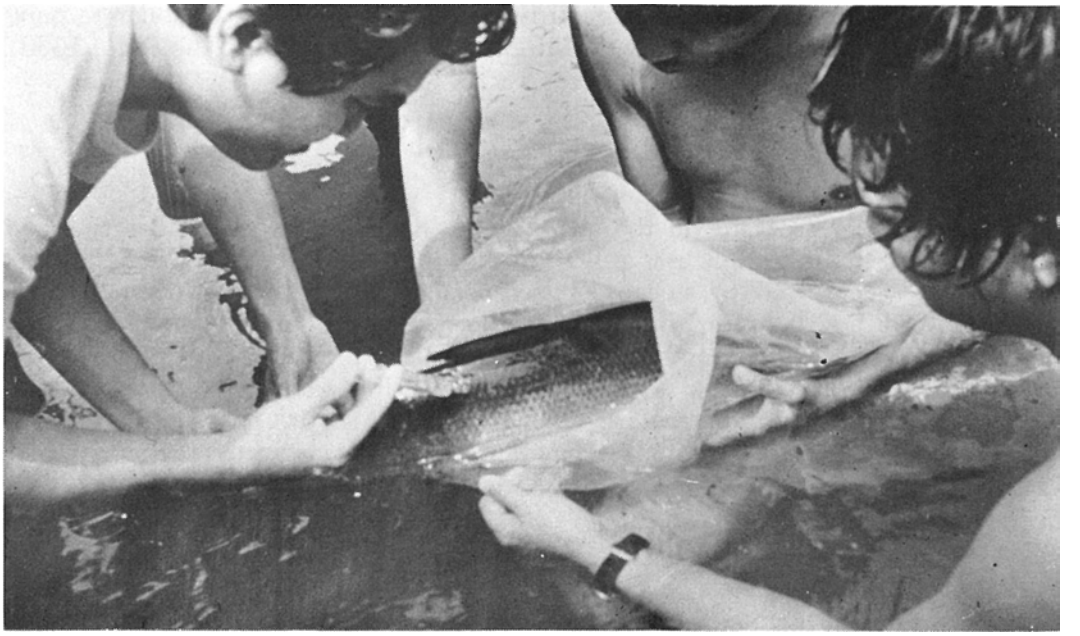


Fig. 5. Intramuscular injection of gonadotropins. Injection is administered a few centimeters below the dorsal fin.

Females

Only fish with eggs having an average diameter of at least 0.65 mm should be induced to spawn. Anesthetize the fish completely by immersing it in seawater containing 100 ppm 2-phenoxyethanol. For the first injection, give the fish a combination of 10 mg SPH/kg body weight + 1,000 IU HCG/kg body weight; the second, 10 mg SPH/kg body weight + 2,000 IU HCG/kg body weight. The time interval between injections is 9-12 hours both for wild and captive milkfish. This interval is used to ensure that final maturation of the oocytes (eggs) is completed before the fish dies or before the eyes become completely covered with an opaque substance. Newly caught fish, if subjected to excessive handling, will die in 2-3 days.

Usually, only 2 injections are needed to induce both captive and wild adult milkfish to spawn, as long as the dosage and time interval mentioned above are followed. However, badly injured fish may need a third injection. In such cases, the third injection should be the same dose as the second. When a third injection is necessary, very often the fish dies before ovulation takes place; and if ovulation does take place, the fertilization and hatching rates are usually very low.

It should be borne in mind that the dosages mentioned here were found to give consistently good results with spawners caught from the wild in water with a salinity of 32 to 35 ppt, and with spawners from the captive stock that were reared to sexual maturity in floating cages in seawater with a salinity of 28 to 35 ppt. Furthermore, the experiments were done at ambient temperature (28°-30°C) and at a salinity of 32 to 34 ppt. The same dosages may not be effective in inducing spawning in fish reared to sexual maturity in earthen ponds or in concrete tanks at lower salinities (Liao and Chen, 1979; Tseng and Hsiao, 1979; Kuo *et al.,* 1980).

Males

One of the problems in the artificial fertilization of eggs has been viscous, non-dispersing milt of milkfish caught during the natural breeding season. This problem can be solved by injecting newly caught males that have viscous milt with 1 ml DF. A day after the injection, the viscous milt will become more fluid and copious and will disperse easily when mixed with seawater; moreover, the percentage of motile sperms as well as sperm motility will increase (Juario *et al.,* 1980). It is more advantageous to use DF rather than HCG because, aside from its longlasting effect which minimizes handling stress, it is cheaper.

Fertilization and incubation

Between 10 and 12 hours (usually 10 hours) after the second injection, the fish will start to spawn eggs. This does not mean, however, that the eggs are already fertilizable. It is still necessary, when the fish starts spawning eggs, to sample the eggs from the gonads by cannulation and to examine them under the microscope. Strip the fish of its eggs only if at least 30%-40% of the eggs are transparent (not glassy transparent); otherwise, insert a plug through the genital pore to prevent further loss of eggs.

Spawning/stripping is done as follows: the holders, two persons with bath towels in their hands, grasp the fish, one holding at the caudal peduncle while the other handles the head region. A third person immediately covers the genital pore with a small towel to prevent further loss of eggs (Fig. 6) He also helps the holders dry the fish. When the fish is dry, turn it upside down and hold the tail down so that the ripe eggs will flow naturally toward the genital pore. The third person holds the spawning basin beneath the genital pore. A fourth person, the spawn taker, gently presses out the eggs with the fingers, beginning pressure just in front of the genital pore (Fig. 7). The hand is then moved nearer the head of the fish and further gentle pressure is applied as necessary to assist the natural flow of eggs until all that will freely come out of the fish are obtained. Collect eggs in separate arbitrary batches. As soon as one batch is collected, fertilize the eggs following the dry method with milt taken by hand-stripping a hormone-treated male. Mix the eggs and milt gently but thoroughly using turkey or chicken feathers. After at least 3 minutes, add seawater (34 ppt salinity) to the mixture while stirring. After another 3 minutes transfer the fertilized eggs to a scoop net (mesh size, not

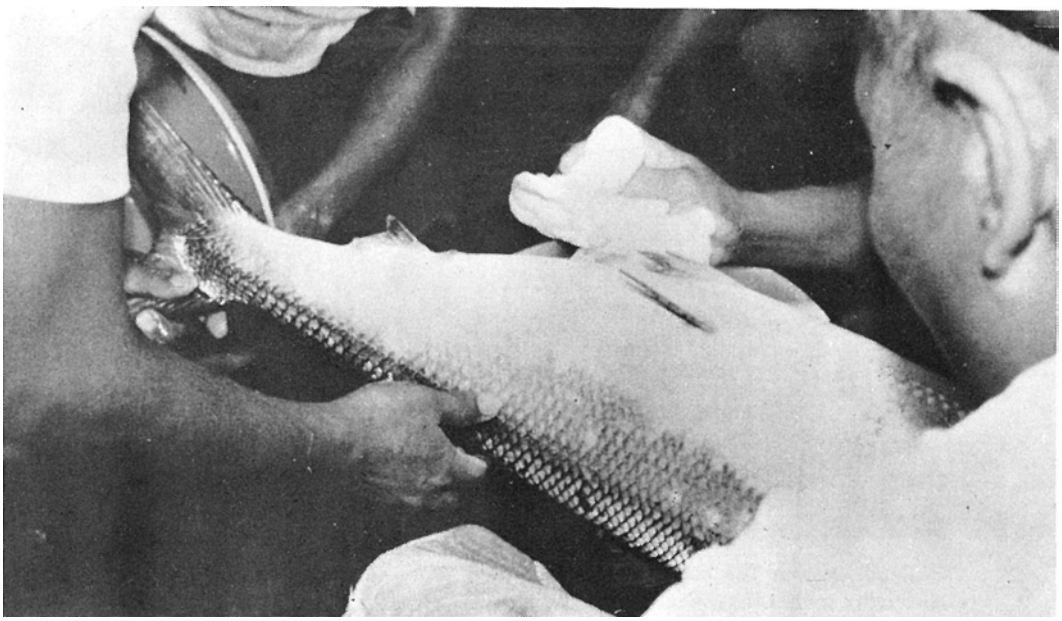


Fig. 6. Genital pore or anal region of the female about to be covered by white cloth to prevent further loss of eggs.

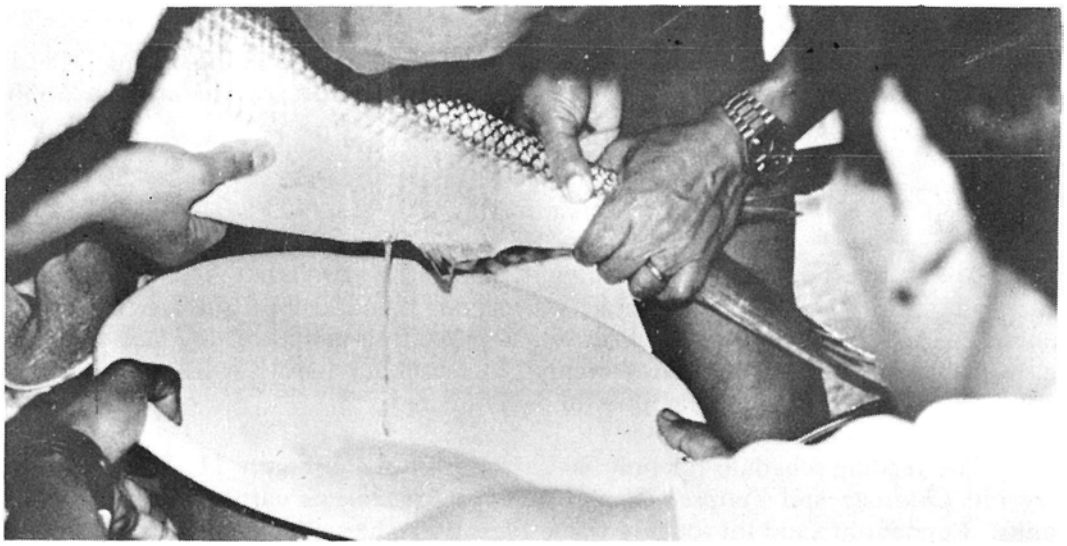


Fig. 7. Stripping the female milkfish of its eggs.

less than 500 microns) and wash the eggs thoroughly with seawater of the same salinity (isohaline) as that in the incubation tanks (Fig. 8). The eggs are then transferred to the incubators. Aerate the incubators strongly to prevent the eggs from clumping and incubate the eggs at ambient temperature (25°-30°C) and at a salinity of 34 ppt. Six hours after the start of incubation, change 1/3 of the water in the tank with new filtered seawater. Remove dead eggs from time to time by siphoning

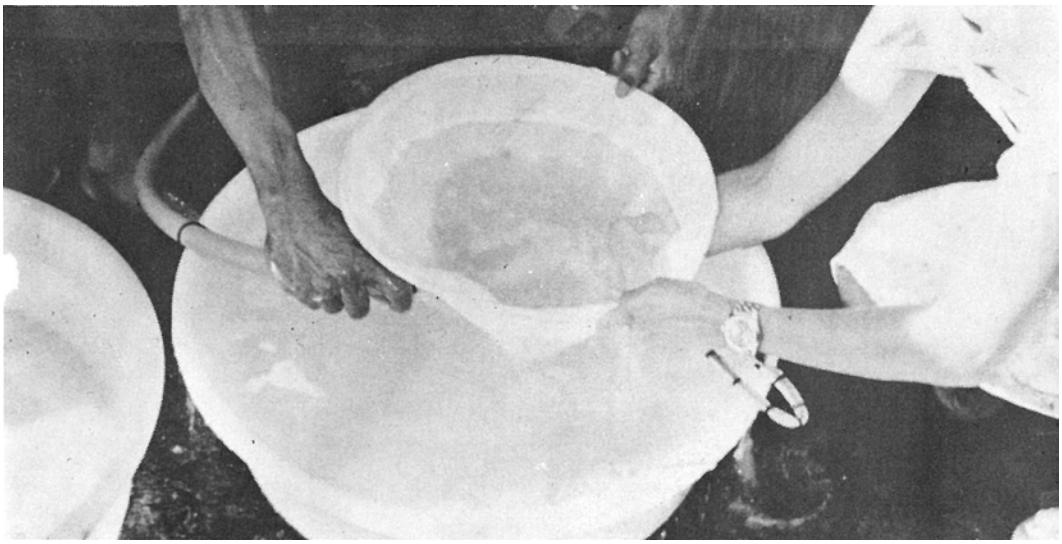


Fig. 8. Fertilized eggs in the scoop net are washed thoroughly with filtered seawater before introducing them into the larval rearing tank.

them out through a polyethylene tube after aeration has been stopped for about 5 min. Fertilized eggs float in seawater with a Salinity of at least 34 ppt while unfertilized eggs sink.

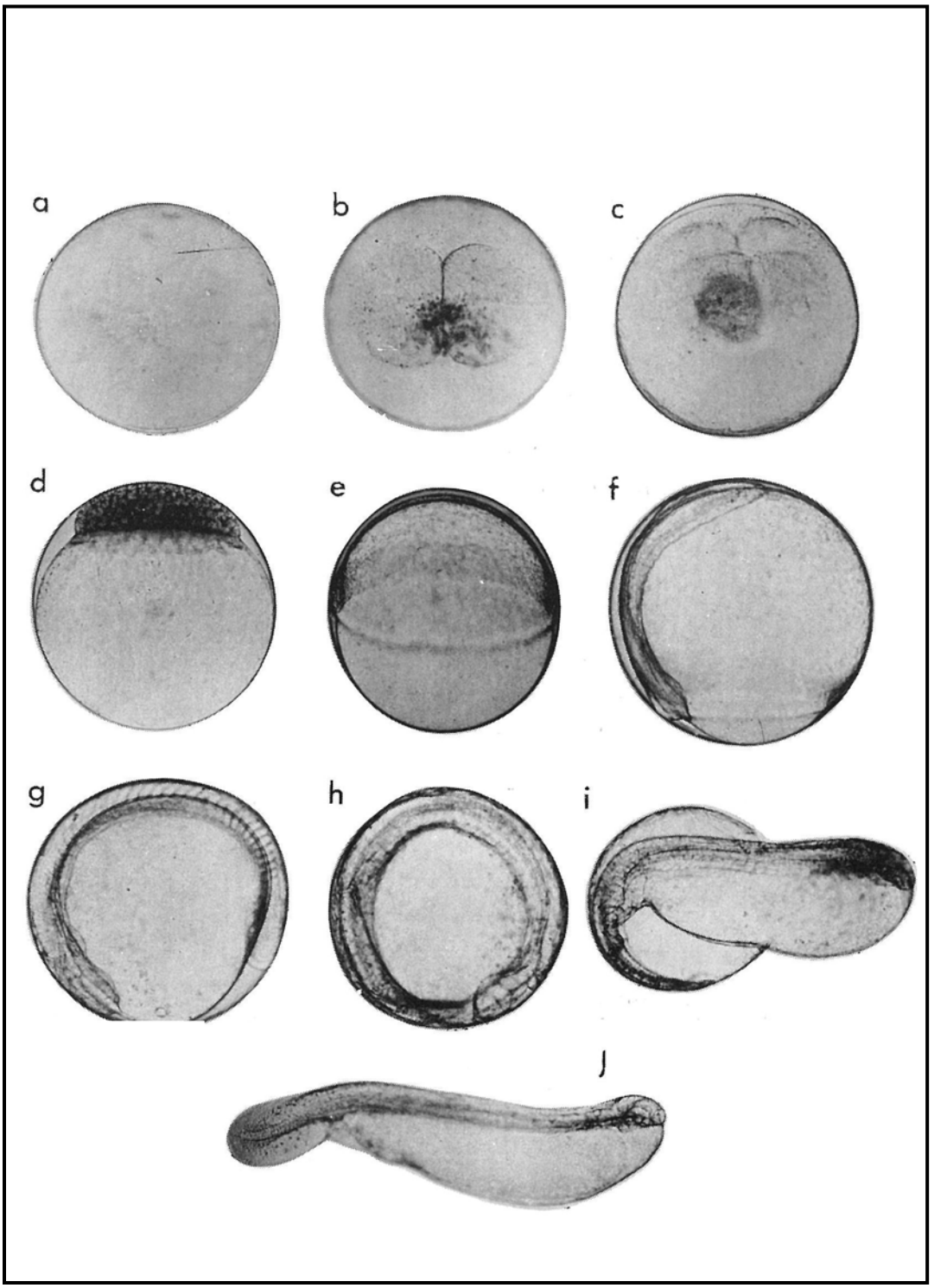
The embryonic development of the milkfish is presented in Fig. 9 and Table 1. Depending upon the ambient temperature at incubation, the eggs will hatch in 24-35 hours.

Larval rearing

About five hours before hatching, transfer the developing eggs to 600-liter fiberglass tanks. Provide the tanks with strong aeration until the larvae start to hatch, i.e., 24-25 hours after fertilization. Each tank should have about 20 developing eggs/liter. When the eggs are hatched, the stocking density will be about 5-10 larvae/liter or more depending upon the hatching rate.

The feeding schedule for milkfish larvae is shown in Figure 11. Add only 4-6 day old *Chlorella* and *Tetraselmis* and 4-day old *Isochrysis* cultures to the rearing tanks. Concentrate and thoroughly wash the rotifers, harpaticoid and newly hatched brine shrimp nauplii with aged, filtered seawater before adding them to the rearing tanks. Decapsulate *Artemia* cysts following the method of Sorgeloos *et al.,* 1977; Appendix 2) before incubation.

Change at least one-third of the water in the rearing tanks with aged and filtered seawater after Day 3 and daily thereafter until Day 21. Provide continuous mild aeration in each rearing tank. If equipment is available, monitor dissolved oxygen, salinity and temperature once a day. Dissolved oxygen should be maintained between 5-6 ppm. Salinity may be gradually lowered to 28 ppt after Day 5.



The embryonic development and hatching of milkfish. a) Fertilized egg b) 2-cell c) 4-cell d) Blastula e) Late gastrula f) Late gastrula; yolk plug and embryonic streak apparent; g) C-shaped embryo h) Late embryonic development i) hatching j) newly hatched larva.

Table 1. The embryonic development and hatching of milkfish at ambient temperature (28°-32°C) and at a salinity of 34 ppt.

TIME AFTER FERTILIZATION (h min)	STAGE OF DEVELOPMENT	PHOTO NO. IN FIG. 9
0.00	Fertilized egg; this is spherical, non-adhesive and transparent; yolk is granulated and has a yellow tinge; no oil globule	a
1.10	2-cell	b
1.16	4-cell	c
5.40	Blastula	d
8.00	Late gastrula; yolk invasion 50% complete	e
10.45	Late gastrula; yolk plug and embryonic streak apparent	f
14.45	Embryonic differentiation; C-shaped embryo with somites; optic and otic vesicles formed	g
21.40	Embryonic differentiation; embryo starts to show twitching movement	h
25.45	Hatching; fully formed embryo emerges head first out of egg shell	i

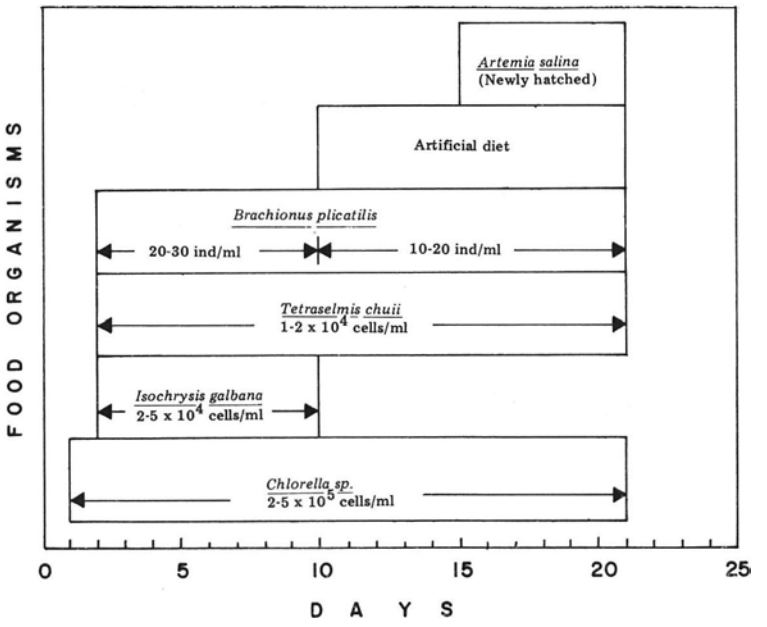


Fig. 10. Feeding schedule for milkfish larvae during the 21-day rearing period

The development and behavior of milkfish larvae are presented in Fig. 11 and 12 and Table 2.

Mass production of larval food

Chlorella sp. and *Tetraselmis chuii*

The following agricultural fertilizers are used for the mass production of *Chlorella* sp. and *Tetraselmis chuii:* (1) Urea (46-0-0) at 10 g/t of filtered seawater (2) Ammonium sulphate (21-0-0) at 100 g/t of filtered seawater (3) Ammonium phosphate (16-20-0) at 10 g/t of filtered seawater.

Dissolve the fertilizers in 5 liters of filtered seawater and broadcast in culture tanks containing filtered seawater and algal starters. The starting density should be about 0.5-1-0 x 10^6 cells/ml for *Tetraselmis* and 0.5-2.0 x 10^6 cells/ml for *Chlorella.* Aerate the culture tanks vigorously throughout the culture period to prevent cells from clumping. The average peak density of 14.0 x 10^6 cells/ml for *Chlorella* and 1.0 x 10^6 cells/ml for *Tetraselmis* is usually attained between days 4 and 8. At this time, harvest the culture and introduce it gradually into the larval rearing tanks. At about day 10 to 12, the population density of *Chlorella* and *Tetraselmis* will start to decline. To ensure a continuous supply of algae, prepare a series of culture tanks to allow daily harvest.

Isochrysis galbana

For the mass production of *I. galbana,* only triple 14 (14-14-14) is used as fertilizer at 40 g/t of filtered seawater. The starting density should be at least 1.0 x 10^4 cells/ml. A peak density of 0.5-1.0 x 10^6 cells/ml is reached between days 3 and 4. At this time, harvest *Isochrysis* and introduce it gradually to the rearing tanks. At about day 4 to 5, the population density of *Isochrysis* will start to decline. If Walne's Medium (Appendix 3) is used to culture *Isochrysis,* an average density of 2.0-3.0 x 10^6 cells/ml is reached between days 5 and 7. The population density of *Isochrysis* will start to decline at about days 8 to 9.

If any of the culture tanks become contaminated, the algal cultures should be discarded immediately. The culture tanks should be disinfected with commercially available "Chlorox" or "Purex", washed and brushed thoroughly and dried before re-use.

Brachionus plicatilis

A density of 2 individuals/ml or even as low as 0.5 individuals/ml is sufficient to start a culture of rotifers in a 1 ton tank containing 100 liters of filtered seawater. Feed rotifers with *Chlorella* at a density of about 1.5-3.0 x 10^5 cells/rotifer/day. Aerate the rotifer culture tanks moderately throughout the culture period. Once the culture tank reaches its full capacity, harvest the rotifers and concentrate by

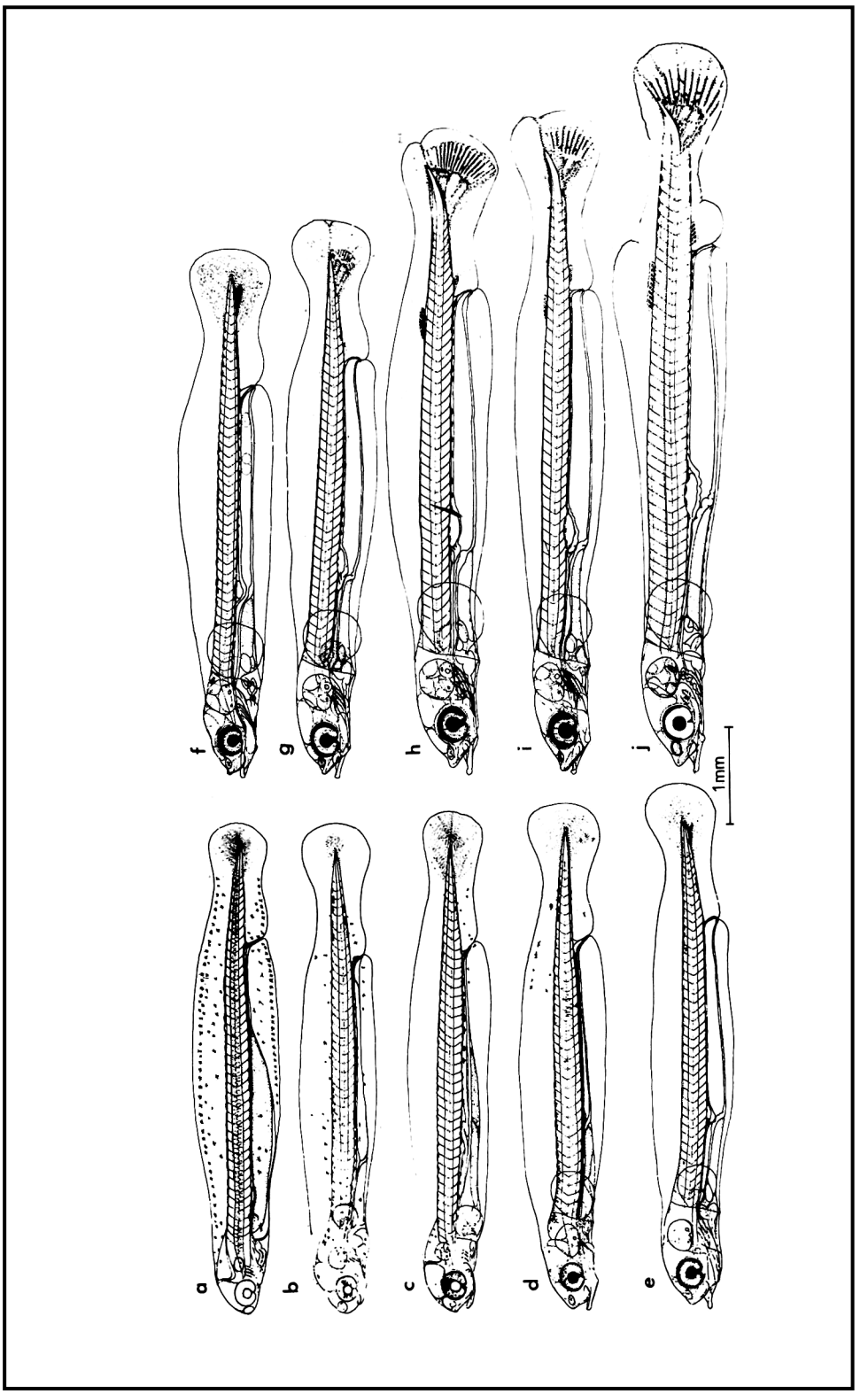


Fig. 11. The development of milkfish larvae. a) 1 day old b) 2 days c) 3 days d) 4 days e) 5 days f) 6 days g) 7 days h) 8 days i) 9 days j) 10 days.

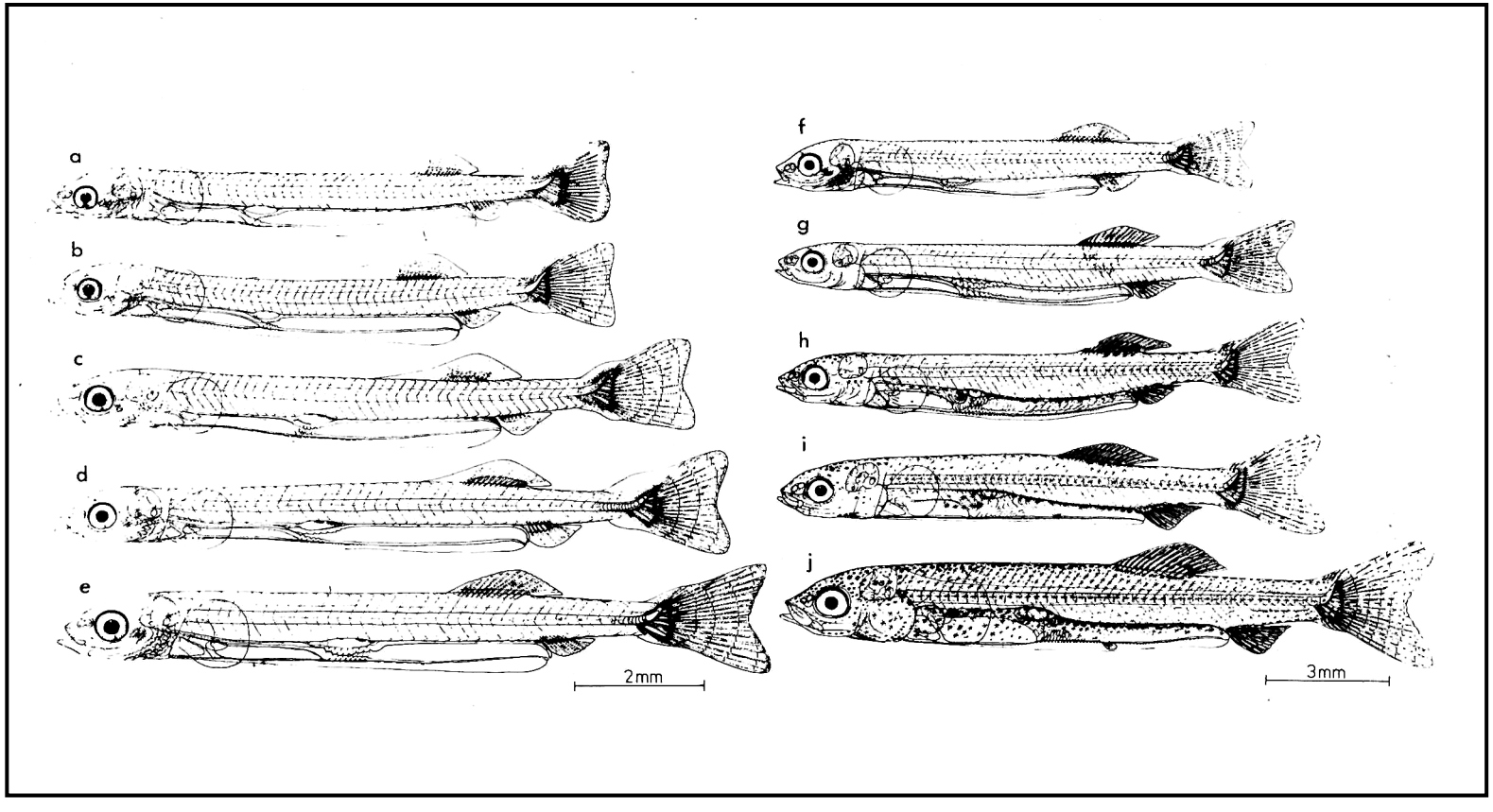


Fig. 12. The development of milkfish larvae (continued). a) 11 days old b) 12 days c) 13 days d) 14 days e) 15 days f) 16 days g) 17 days h) 18 days i) 19 days j) 21 days.

Table 2. The development and behavior of milkfish larvae.

DAYS AFTER HATCHING	MEAN TOTAL LENGTH(mm)	FIG. NO.	DEVELOPMENT AND BEHAVIOR
0	4.27 + 0.11	11	Newly hatched larva slightly curved with unpigmented eyes; mouth not formed, anus closed and located posterior to the yolk mass; yolk sac broad and extends anteriorly near the head end of the larva. Pigments sparsely scattered in the yolk mass and on the head end of the larva. Larva remains suspended in the water column with head down and belly up and sink slowly in an oblique position; then it makes a quick 360° upward turn and swims to the surface.
1	5.14 + 0.11	12a	Eyes still unpigmented; yolk sac is reduced; pectoral fins start to develop; mouth and anus still closed.
2	5.18 + 0.12	12b	Eyes starting to be pigmented; mouth and anus opened; yolk very much reduced.
3	5.22 + 0.12	12c	Eyes well pigmented; yolk completely absorbed; feeding behavior apparent. The larvae exhibit phototaxis during the day but drift at night.
4 to 5	5.29 + 0.26 5.31 + 0.24	12d 12e	Heart chambers well developed; healthy larvae feed actively. Critical period starts at Day 4.
6 to 7	5.51 + 0.24 5.62 + 0.46	12f 12g	Pectoral fins well developed; a bilobal caudal fin becomes apparent; critical period ends at Day 7.
8 to 9	6.33 + 0.42 6.38 + 0.14	12h 12i	Operculum is starting to form; growth starts to accelerate.

DAYS AFTER HATCHING	MEAN TOTAL LENGTH (mm)	FIG. NO.	DEVELOPMENT AND BEHAVIOR
10	6.72 + 0.42	12j	Dorsal and anal fin start to differentiate; caudal fin more differentiated; body becomes very transparent; larvae swim in school and show strong rheotaxis during the day.
11	7.52 + 0.41	13a	Dorsal and anal finfold separate from caudal finfold; body transparent and assumes the pigmentation pattern of wild fry.
12 to 13	7.00 + 0.57 7.96 + 0.85	13b 13c	Caudal finfold is separated completely from the dorsal and anal finfolds; pigmentation on the dorsal portion of trunk increases; but it is reduced on the dorsal peritoneal membrane except in the region above the air bladder and the posterior portion of the anus; variability in size of larvae becomes apparent.
14 to 15	8.26 + 1.15 10.51 + 0.73	13d 13e	Fin development resembles that of wild fry; larvae swim circularly and rapidly during the day; larvae show strong phototaxis at night.
16 to 17	10.67 + 0.66 11.87 + 0.66	13f 13g	Pigmentation on head region increases; intestinal wall folds increase and become distinct; rudimentary gill rakers apparent; larvae are very active and react to sudden movements.
18 to 19	12.44 + 0.63 13.36 + 0.53	13h 13i	Intestinal folds distinct; rudimentary pelvic fins apparent; pigmentation pattern differs from that of wild fry; larvae are no longer sensitive to direct exposure to sunlight; larvae feed on algae growing on the wall of rearing tank.

DAYS AFTER HATCHING	MEAN TOTAL LENGTH(mm)	FIG. NO.	DEVELOPMENT AND BEHAVIOR
20	13.63 + 0.27		
21	14.09 + 0.60	13j	Pelvic fins developing; pigments scattered over the upper half of the body and dense on the peritoneal wall; larvae now resistant to handling and could be transported to and stocked in nursery ponds.

filtration through a bolting cloth with a mesh size of 150 and 48 microns. As in the mass culture of *Chlorella,* prepare a series of culture tanks so that there is sufficient food for the fish larvae throughout the rearing period.

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Appendix 1. Assessment of sperm motility (Mounib, 1978)

Sperm motility is assessed in two ways: (1) The number of motile spermatozoa is expressed as a percentage of the total number of spermatozoa; and (2) the degree of progression of the motile spermatozoa is calculated on a scale of 0-10 in which 1 refers to a very feeble spermatozoon and 10 refers to a spermatozoon with good progressive motility.

Appendix 2. Decapsulating Artemia cysts (Sorgeloos *et al.,* 1977)

1. Dry cysts are hydrated in a funnel-shaped container with tap water or sea water and kept in continuous suspension by aeration from the bottom.

2. After 1 hr, the suspension is diluted with an equal volume of commercial hypochlorite to obtain a final concentration of active ingredients of 2.12% (oxidation process starts immediately and, as the chorion dissolves, a gradual colour change is observed in the cysts from dark brown via white to orange). The decapsulation processes must be kept below 40°C.

3. Within 7-10 min, the chorions disappear completely and the decapsulated cysts should then be filtered immediately and thoroughly washed with tap water or sea water in order to remove all traces of hypochlorite.

4. The treated cysts are now either incubated directly for hatching or, after immediate dehydration in a brine solution, stored for later use.

Appendix 3: Walne's Medium (Walne, 1974)

Solution A

$NaNO_3$	100.00 g
Na_2EDTA	45.00 g
H_3BO_3	33.60 g
$NaH_3PO_4 \cdot 2H_2O$	20.00 g
(or $NaH_3PO_4 \cdot H_2O$)	17.69 g
$FeCl_3 \cdot 6H_2O$	1.30 g
$MnCl_2 \cdot 4H_2O$	0.36 g

Dissolve Solution A in 1 liter of distilled water.

Solution B (Trace metal stock)

$ZnCL_2$	2.1 g
$CoCl_2 \cdot 6H_2O$	2.0 g
$(NH_4)_6MO_7O_{24} \cdot 4H_2O$	0.9 g
$CuSo_4 \cdot 5H_2O$	2.0 g

Dissolve Solution B in 100 ml of distilled water and acidify using 1N HCl until the solution clears. Use 2 ml of Solution B per liter of Solution A

Solution C (Vitamin Stock Solution)

B_{12} (Biotin)	10.0 mg
B_1 (Thiamine)	200.0 mg

Dissolve Solution C in 200 ml of distilled water. Use 0.1 ml of Solution C per liter of Solution A.

N.B.

Walne's Medium is given at a rate of 1 ml per liter of culture.

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